NUCLEUS OF CHILOMONAS PARAMŒCIUM EHRENBERG.

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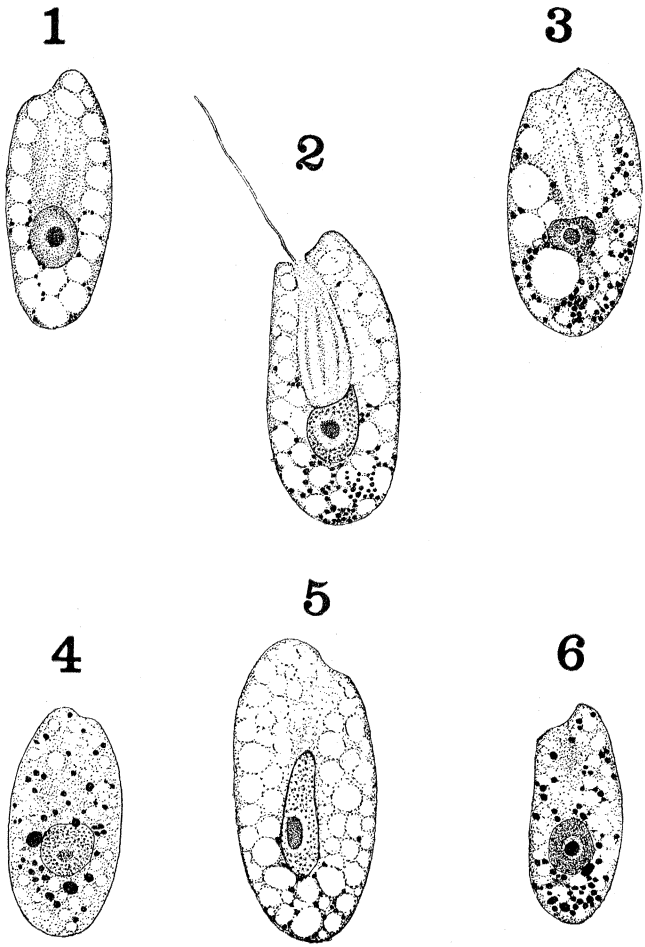
Chilomonas paramœcium Ehrenberg is perhaps the most common flagellate to be found in laboratory aquaria; but the nature of our paper demands that we describe in detail the species with which we are dealing and our methods of preparation.

Our specimens were found in dense swarms in laboratory aquaria that had stood for a certain time. The specimens were taken from the aquarium with a pipette and centrifuged. The infusion-water was then decanted off and the animals were next fixed in aceto-sublimate for one to three minutes and then washed and passed into 70 per cent. alcohol and stained in Delafield's hæmatoxylin. The stained specimens were mounted in dammar. The length of the fixed animals was as much as thirty microns. In life the posterior end of the animal is somewhat pointed and slightly bent dorsally. The anterior end is obliquely truncated from anterior dorsal region posteriorly to ventral side. A pharynx leads from the anterior oblique end of the body well towards or even beyond its middle (Figs. 1 and 2). Associated with this pharynx are certain small rounded bodies arranged in eight rows, each row lying nearly parallel to the axis of the body. These bodies in life appear as highly refractive structures embedded within the wall of the pharynx. Traces of these are not always found in our fixed material. They appear as concentric, arched lines lying parallel to the axis of the body when our fixed material does show them (Fig. 2). Pascher ('13) described these bodies as "kugeligen Körnchen . . . Diese Körnchen entsprechenden der Körnenauskleidung der Furche der niederen Kryptomonaden und rudimentare 'Trichocysten' dar." Bělař ('16) gives a figure of discharged Tapetenkörner or Trickocysten and says: "es scheint hier vielmehr um mehr oder weniger funktionslos gewordene Rudimente der Schleimtricko-

cysten der Cryptomonaden zu handeln." There are also associated with the pharynx two flagella of equal length. Eyferth ('00) says that these arise from the dorsal wall of the pharynx; Jennings ('06, Fig. 72) in his figure does not commit himself as to the place of origin of the flagella. Calkins ('01, Fig. 10, *B*) and Pascher ('13, Fig. 171) show one flagellum arising from the dorsal and the other from the ventral side of the pharynx. A contractile vacuole is within the dorsal anterior extremity of the body. The general cytoplasm is not alveolar, though it usually presents such appearance, because of the presence of colorless, highly refractive spheroidal bodies. It is important to note that these bodies vary in number, and in so far as they decrease in number the apparent alveolar condition of the cytoplasm becomes less evident. We have tested these with the iodine starch test and got a negative reaction—the bodies staining brown. These bodies have the same general optical features as the paramylum grains of *Euglena* and have been described for *Chilomonas* as paramylum grains; they are, therefore, to be looked upon as assimilation products. These assimilation products are more or less directly related to certain deeply staining (nuclear stains) rounded bodies, which lie about them.

Most systematic and experimental workers, when describing *Chilomonas paramœcium* have paid little or no attention to these chromatic bodies. For example, Eyferth ('00), Pascher ('13) and Jennings ('06) neither describe nor depict them. These same authors, however, describe a centralized well-defined nucleus lying behind the middle of the body.

Calkins ('99) recognized no nucleus in this rounded, nuclear-like structure, which lay behind the middle of the body; but he looked upon the deeply staining bodies which lie by the paramylum grains as a distributed nucleus, and thus brought *Chilomonas paramœcium* in line with a series of animals bearing distributed nuclei or permanently granular nuclei. "Forms with this permanently granular chromatin, again, are found in two conditions. In one type the granules are scattered throughout the entire cell, and are never confined by a nuclear membrane (so-called 'distributed' or 'diffused' nuclei). In nuclei of the other type the granules are confined in a definite, more or less



FIGS. 1 AND 5. Specimens with closely packed paramylum grains and few chromatic bodies. × 1,500.

FIG. 2. Shows ventral flagellum, pharynx with rows of structures within its wall—the rudimentary trichocysts. Nucleus is here closely applied to the fundus of the pharynx. Paramylum grains reduced somewhat and the number of chromatic bodies is increased. × 1,500.

FIGS. 3, 4 AND 6. Show the paramylum grains greatly reduced in size and the chromatic bodies greatly increased in size. In these cases the size of both paramylum grains and chromatic bodies varies much. × 1,500.

spherical space, which may or may not be bounded by a nuclear membrane. Examples of the first type were described by Gruber in certain Rhizopods and in a number of Ciliata, and,

as he suggested, it is highly probable that many, if not all of Haeckel's Monera will be found to possess nuclei of this type. Among flagellated forms it has been described by Bütschli ('96) for *Chromatium okenii* and *Ophidomonas jenensis*, and by myself ('98) for a species of *Tetramitus*. In the latter form the granules of chromatin, which at first are scattered throughout the entire cell with no apparent order, come together to form a loose aggregate prior to division. In this condition the aggregate is divided into halves, an equal portion going to each daughter-nucleus (Fig. 2). It is important to note here, however, that another element comes in to complicate the process. In the resting condition of the cells, when the chromatin is distributed throughout the cytoplasm, a faintly staining body can be found somewhere near the center of the cell (Fig. 2, *A*). This body becomes more definite as the chromatin granules come together for division, and it divides into equal portions before the nucleus is halved. During the process of division the chromatin granules become heaped about this partly divided body, one half of which remains in the center of each daughter-heap of granules until the end of the division (Fig. 2, *D–E*). After division the granules again separate, forming the distributed nucleus. The central body, therefore, has the attributes of an attraction sphere.

"The chromatin represented by this temporary aggregation of chromatin granules about the sphere is permanent in the majority of the Flagellates, and may perhaps be regarded as the usual condition of protozoan nuclei. Among some Flagellates the aggregation of chromatin granules about the sphere, although permanent throughout resting and active phases, resembles the loose aggregation of the division period of *Tetramitus* in having no nuclear membrane (*Chilomonas paramœcium*, *Trachelomonas lageuella* and *T. hispida*)." Again Calkins ('01) speaks of what others take to be a nucleus as a division center: "The flagellate *Tetramitus* shows an apparently similar division-center. During the resting phases, the chromatin is distributed throughout the cell, while an indefinite 'achromatic mass' appears to be in direct connection with the cytoplasmic reticulum. Immediately before division, however, the chromatin granules collect about this body, and then, save for the absence

of a membrane, the aggregate resembles the nucleus of *Euglena*. Division takes place as in *Euglena*, the intranuclear division-center dividing first. After division the chromatin granules again disperse and the division-center becomes again cytoplasmic (Fig. 143)." "An intermediate stage between this condition and the condition in *Euglena* is shown by some species of *Chilomonas* and *Trachelomonas* in which there is no nuclear membrane, but in which the chromatin remains permanently aggregated about the division-center." Kellicott ('13), in his statement "a definite nuclear membrane may be absent at first, as in *Chilomonas*," seems to follow Calkins's earlier conception that these granules represent a nucleus without a nuclear membrane.

It is interesting to see that Calkins ('08) modifies his viewpoint somewhat. He no longer considers these granules as representing a nucleus, but implies that they are nuclear in origin. Though it is not exactly clear that he has changed his opinion about these chromatin granules such is strongly implied when he says "there is probably no great difference between the above-described method of idiochromidia formation by transfusion, whereby the chromatin materials percolate through the nuclear membrane in fluid form, and that by nuclear dissolution, whereby the peripheral portion of the nucleus becomes scattered in granular form throughout the cell body" and then in his legend to Fig. 49 on the same page prints "*Chilomonas paramœcium* to show the alveolar structure of protoplasm prior to idiochromidia formation." This idiochromidia he at this place considers to be the "sexual or racial chromatin" of the cell as contrasted with chromidia which is "functionless extranuclear chromatin."

By the implication of his later words he has given up the idea that the nuclear-like body at the base of the pharynx is a nucleus. He now sees, in what he had taken to be the chromatin granules of a distributed nucleus, grains of idiochromidia. These, he says, are of nuclear origin. He further thinks that these chromatic granules play an important part in reproduction.

Our own observations indicate that there is a definite or clearly defined nucleus in *Chilomonas paramœcium*. This

nucleus lies posterior to the middle of the body closely associated with the fundus of the pharynx (Figs. 2 and 3). We have been able to find many of these nuclei in what we take to be the resting condition (Fig. 3). In this condition there is a well-defined nuclear limit indicating the presence of a nuclear membrane. Within the nucleus a single spheroidal nucleolus is seen. This nucleolus in life is highly refractive. The chromatin granules of the resting nucleus are very small and uniform in size. They crowd rather closely upon the nucleolus and leave the nucleolus to lie within a relatively small chromatin-free region (Figs. 3 and 6). In other specimens we find the nuclear size to be increased (Fig. 2) and in some cases the shape of the nucleus, as well, is greatly modified (Fig. 5). In most of these enlarged nuclei the chromatin granules lie more remote from the latter within a relatively large chromatin-free region. The chromatin granules themselves, in such nuclei, are greatly enlarged, though yet uniform in size. These enlarged nuclei we had taken to be prophases in mitosis before the appearance of Bělař's paper ('16) and now we can say that we are able to corroborate in a general way his earlier details for the mitosis of *C. paramœcium*.

Except for these strikingly nuclear-like variations, the character, of what has been termed a "division center," remains constant and we look upon it as the proper nucleus of the cell.

On the other hand, the variability of the extra-nuclear chromatic granules is conspicuous in contrast with the slight variability of the nucleus. These chromatic granules vary in number, size and distribution. We have not found an individual in which no granules are present. These chromatic bodies are smallest when fewest and they then lie within the posterior half of the cell (Figs. 1 and 5). The granules tend to increase in size as they become more numerous (Figs. 2, 3 and 6). They are most widely distributed throughout the cell when they are largest (Figs. 4 and 6). The granules of a given cell, when they are in this last condition, are no longer uniform in size (Figs. 4 and 6).

Associated with this variability of the chromatic bodies we have a variability of the paramylum contents of the cytoplasm. The granules are fewest and smallest when the paramylum bodies are relatively most numerous and when the latter lie

closely packed within the cytoplasm (Figs. 1 and 5). As the paramylum granules become reduced in size the chromatic bodies become more frequent and lie within the enlarged masses of cytoplasm that fill the interstices between the paramylum grains (Figs. 2 and 3). Finally the granules of chromatic material are largest, most numerous and most widely distributed when the paramylum grains have been reduced to small, widely separated spheroids (Figs. 4 and 6).

These chromatic bodies, moreover, arise in the cytoplasmic interstices between the paramylum grains. They lie closely applied to the paramylum elements rather than to the nucleus. Their contact with the nucleus seems to be purely incidental. They appear rather to be bodies related to the changes involved in the assimilation of the paraymlum grains. Their origin, then, we consider to be cytoplasmic and not nuclear. These chromatic granules we take to represent by-products or intermediate phases of the assimilation of paramylum and are not idiochromidia.

Conclusions.

1. There is a well-defined nucleus with a definite nuclear membrane in *Chilomonas paramœcium.*

2. The chromatic bodies, that are found within the cytoplasm, are related to the formation and disposal of paramylum and must not be considered to be idiochromidia, for they are cytoplasmic and not nuclear in origin.

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